using a Coulter Counter. As shown in Figure 2, the dendritic nitric oxide donor enhanced endothelial cell growth and inhibited smooth muscle cell proliferation. These experiments have been repeated with varying doses of NO, and proliferation was quantified using immunohistochemical staining for proliferating cell nuclear antigen, or PCNA, which stains cells in the S-phase of mitosis.

The affect of a dendritic nitric oxide donor on platelet aggregation was studied as follows. Blood was obtained from a healthy volunteer with 10 U/mL heparin (Sigma Chemical Co., St. Louis, MO). 10 μM mepacrine (Sigma Chemical Co., St. Louis, MO) was added for 20 minutes at 37°C to fluorescently label the platelets. Glass slides were incubated with collagen I in 3% glacial acetic acid in distilled water (2.5 mg/mL) for 45 minutes in a humidified chamber at room temperature, and then rinsed gently with PBS. Labeled blood was incubated, for 30 minutes at 37°C, with either (i) the dendritic nitric oxide donor of the present invention that comprised an eight-arm PEG core, a 3rd generation branching unit, and NO, or (ii) a control that comprised the same compound without NO. The slides were then rinsed with PBS to remove all visible blood. The number of adherent platelets per field of view (200X) was determined using a fluorescent microscope (Zeiss Axiovert 135, Thornwood, NY). As seen in Figures 3 and 4, the dendritic nitric oxide donor was able to inhibit platelet adhesion to collagen-coated slides (12.3 ± 4.5 platelets per field of view) as compared to platelets exposed to the control (64.6 ± 7.5 platelets per field of view, p <0.00000005).

Stimulation of endothelial cell proliferation and inhibition of smooth muscle cell proliferation by targeting certain dendritic nitric oxide donors.

To demonstrate targeting, a dendritic nitric oxide donor conjugated to fluorescently labeled sialyl-Lewis-X was synthesized and studied as follows (Figure 8). Lysine dendrons were reacted with fluorescein 5-isothiocyanate in dimethyl sulfoxide (DMSO) to fluorescently label the dendrons. Other dendrons were reacted with biotin- NHS for later conjugation of sialyl-Lewis-X-biotin using avidin as a linker, while others were reacted with NO gas in water. FITC conjugated dendrons, biotinylated dendrons, and NO-releasing dendrons were reacted with multi-armed PEG to form a species having a

fluorescent tag and available biotin to bind the targeting molecule. Sialyl-Lewis-X was reacted with avidin in water, and then added to a solution of FITC-labeled biotinylated dendrimers to allow binding of sialyl-Lewis-X to the dendrimers.

The dendritic nitric oxide donor having a fluorescently labeled sialyl-Lewis-X was then studied as follows. Human umbilical vein endothelial cells (HUVECs) were seeded in 6-well tissue culture plates at 20,000 cells/cm^2 and allowed to adhere for 24 hours. Cells were incubated with 5 μg/mL Interleukin-1β for 4 hours at 37°C, then exposed to either FITC-labeled sialyl-Lewis-X conjugated dendrimers (Figure 9A) or FITC-labeled non-targeted dendrimers (Figure 9C) for 30 minutes. As negative controls, a portion of the cells was not activated, and thus did not display elevated levels of E-selectin, and another set of cells were exposed to an E-selectin antibody after activation (Figure 9B). The cells were then rinsed 3 times with PBS to remove non-adherent dendrimers and examined by fluorescence microscopy to determine the extent of binding. As shown in Figure 9, the sialyl-Lewis-X conjugated dendritic nitric oxide donors preferentially bind HUVECs.

Therefore, the present invention is well adapted to attain the ends and advantages mentioned as well as those that are inherent therein. While numerous changes may be made by those skilled in the art, such changes are encompassed within the spirit of this invention as defined by the appended claims.

What is claimed is:

1. A dendritic nitric oxide donor having the formula:

$$[P]-[(A)_y]_x-[(NO)_z]_q$$

wherein P is a core that comprises a biocompatible polymer;

A is a branching unit monomer that comprises at least one end group capable of reversibly attaching NO;

(NO) is nitric oxide;

x, y, and z are positive integers greater than or equal to 1; and

q is a positive integer greater than or equal to y.

2. The dendritic nitric oxide donor of claim 1 wherein the dendritic nitric oxide donor comprises a metabolically produced form of the dendritic nitric oxide donor.

3. The dendritic nitric oxide donor of claim 1 wherein P comprises a functional group.

4. The dendritic nitric oxide donor of claim 1 wherein P comprises a functional group, wherein the functional group chosen from the group consisting of an amine group, a hydroxyl group, a N-hydroxysuccinimide ester, a carboxyl group, and combinations thereof.

5. The dendritic nitric oxide donor of claim 1 wherein P further comprises NO.

6. The dendritic nitric oxide donor of claim 1 wherein P comprises a metal.

7. The dendritic nitric oxide donor of claim 1 wherein P is chosen from the group consisting of polyethylene glycol, poly(ethylenamine), poly(amidoamine), polypropylenimine tetraamine, and a combination thereof.

8. The dendritic nitric oxide donor of claim 1 wherein P is chosen from the group consisting of methoxypoly(ethylene glycol)-amine, diaminopoly(ethylene glycol), PEG-N-hydroxysuccinimide ester monoacrylate, a multi-arm PEG, an mPEG-NHS, and a combination thereof.

9. The dendritic nitric oxide donor of claim 1 wherein A further comprises a functional group capable of releasing NO.

10. The dendritic nitric oxide donor of claim 1 wherein A further comprises a functional group capable of releasing NO chosen from the group consisting of an amine group, a carboxyl group, a thiol group, a hydroxyl group, and a combination thereof.

11. The dendritic nitric oxide donor of claim 1 wherein the end group of A is chosen from the group consisting of a primary amine, a thiol, a ferrous nitro complex, an organic nitrite, a nitrate, and a combination thereof.

12. The dendritic nitric oxide donor of claim 1 wherein the end group of A is capable of forming a NO-nucleophile complex.

13. The dendritic nitric oxide donor of claim 1 wherein the end group of A is capable of forming diazeniumdiolate ion.

14. The dendritic nitric oxide donor of claim 1 wherein the end group of A is capable of forming a NO-donating group.

15. The dendritic nitric oxide donor of claim 1 wherein the end group of A is capable of forming an *S*-nitrosothiol.

16. The dendritic nitric oxide donor of claim 1 wherein the end group of A is capable of forming a chemical species chosen from the group consisting of organic nitrites and nitrates, ferrous nitro complexes, sydnonimines, and combinations thereof.

17. The dendritic nitric oxide donor of claim 1 wherein A comprises an amino acid.

18. The dendritic nitric oxide donor of claim 1 further comprising a targeting agent.

19. The dendritic nitric oxide donor of claim 1 further comprising a targeting agent chosen from the group consisting of a protein, an antibody, an antibody fragment, a peptide, a cytokine, a growth factor hormone, a lymphokine, a nucleic acid that binds corresponding nucleic acids through base pair complementarity, a cellular receptor-targeting ligand, a fusogenic ligand, a nucleus-targeting ligand, an integrin receptor ligand, molecules that bind to a cell surface molecule, folic acid, and a combination thereof.

20. The dendritic nitric oxide donor of claim 1 further comprising a targeting agent that is capable of binding to a selectin.

21. The dendritic nitric oxide donor of claim 1 further comprising a targeting agent comprising sialyl-Lewis-X.

22. The dendritic nitric oxide donor of claim 1 further comprising a guest molecule.

23. The dendritic nitric oxide donor of claim 1 further comprising a guest molecule chosen from the group consisting of a drug, a therapeutic agent, a diagnostic agent, and a combination thereof.

24. The dendritic nitric oxide donor of claim 1 further comprising a guest molecule comprising 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole.

25. A kit comprising at least one dendritic nitric oxide donor, wherein the dendritic nitric oxide donor comprises a compound having the formula:

$$[P]-[(A)_y]_x-[(NO)_z]_q$$

wherein P is a core that comprises a biocompatible polymer;

A is a branching unit monomer that comprises at least one end group capable of reversibly attaching NO;

(NO) is nitric oxide;

x, y, and z are positive integers greater than or equal to 1; and

q is a positive integer greater than or equal to y.

26. The kit of claim 25 further comprising a drug, a therapeutic agent, a diagnostic agent, or a combination thereof.

27. The kit of claim 25 wherein the dendritic nitric oxide donor further comprises a targeting agent.

28. The kit of claim 25 wherein the dendritic nitric oxide donor further comprises a targeting agent chosen from the group consisting of a protein, an antibody, an antibody fragment, a peptide, a cytokine, a growth factor hormone, a lymphokine, a nucleic acid that binds corresponding nucleic acids through base pair complementarity, a cellular receptor-targeting ligand, a fusogenic ligand, a nucleus-targeting ligand, an integrin receptor ligand, molecules that bind to a cell surface molecule, folic acid, a selectin ligand, sialyl-Lewis-X, and a combination thereof.

29. The kit of claim 25 wherein the dendritic nitric oxide donor further comprises a guest molecule.

30. The kit of claim 25 wherein wherein P is chosen from the group consisting of polyethylene glycol, poly(ethylenamine), poly(amidoamine), polypropylenimine tetraamine, methoxypoly(ethylene glycol)-amine, diaminopoly(ethylene glycol), PEG-N-hydroxysuccinimide ester monoacrylate, a multi-arm PEG, an mPEG-NHS, and a combination thereof.

31. The kit of claim 25 wherein A comprises an amino acid.

32. The kit of claim 25 further comprising a delivery means.

33. The kit of claim 25 further comprising a delivery means chosen from the group consisting of a syringe, an inhaler, pressurized aerosol canister, and a combination thereof.

34. The kit of claim 25 further comprising a container means.

35. The kit of claim 25 further comprising a container means chosen from a vial, a test tube, a flask, bottle, a syringe, and a combination thereof.

36. A medical device comprising at least one dendritic nitric oxide donor, wherein the dendritic nitric oxide donor comprises a compound having the formula:

$$[P]-[(A)_y]_x-[(NO)_z]_q$$

wherein P is a core that comprises a biocompatible polymer;

A is a branching unit monomer that comprises at least one end group capable of reversibly attaching NO;

(NO) is nitric oxide;

x, y, and z are positive integers greater than or equal to 1; and

q is a positive integer greater than or equal to y.

37. The medical device of claim 36 wherein the dendritic nitric oxide donor further comprises a targeting agent.

38. The medical device of claim 36 wherein the dendritic nitric oxide donor further comprises a targeting agent chosen from the group consisting of a protein, an antibody, an antibody fragment, a peptide, a cytokine, a growth factor hormone, a lymphokine, a nucleic acid that binds corresponding nucleic acids through base pair complementarity, a cellular receptor-targeting ligand, a fusogenic ligand, a nucleus-targeting ligand, an integrin receptor ligand, molecules that bind to a cell surface molecule, folic acid, a selectin ligand, sialyl-Lewis-X, and a combination thereof.

39. The medical device of claim 36 wherein the dendritic nitric oxide donor further comprises a guest molecule.

40. The medical device of claim 36 wherein the dendritic nitric oxide donor further comprises a guest molecule comprising 3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole.

41. The medical device of claim 36 wherein the dendritic nitric oxide donor further comprises a guest molecule chosen from the group consisting of a drug, a therapeutic agent, a diagnostic agent, and a combination thereof.

42. The medical device of claim 36 wherein P is chosen from the group consisting of polyethylene glycol, poly(ethylenamine), poly(amidoamine), polypropylenimine tetraamine, methoxypoly(ethylene glycol)-amine, diaminopoly(ethylene glycol), PEG-N-hydroxysuccinimide ester monoacrylate, a multi-arm PEG, an mPEG-NHS, and a combination thereof.

43. The medical device of claim 36 wherein A comprises an amino acid.

44. The medical device of claim 36 wherein the dendritic nitric oxide donor is incorporated into a hydrogel.

45. The medical device of claim 36 wherein the dendritic nitric oxide donor is incorporated into a hydrogel comprising a polymer chosen from the group consisting of poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), and combinations thereof.

46. The medical device of claim 36 wherein the medical device is chosen from the group consisting of a suture, a vascular implant, a stent, a heart valve, a drug pump, a drug-delivery catheter, an infusion catheter, a drug-delivery guidewire, an implantable medical device, and combinations thereof.

47. A method of delivering nitric oxide into a recipient subject comprising:

providing a dendritic nitric oxide donor having the formula:

$$[P]-[(A)_y]_x-[(NO)_z]_q$$

wherein P is a core that comprises a biocompatible polymer;

A is a branching unit monomer that comprises at least one end group capable of reversibly attaching NO;

(NO) is nitric oxide;

x, y, and z are positive integers greater than or equal to 1;

q is a positive integer greater than or equal to y; and

administering the dendritic nitric oxide donor into a recipient subject, such that the dendritic nitric oxide donor releases NO in the recipient subject.

48. The method of claim 47 wherein the dendritic nitric oxide donor has a metabolically produced form.

49. The method of claim 47 wherein the recipient subject has a cardiovascular disease or condition.

50. The method of claim 47 wherein the recipient subject has a cardiovascular disease or condition chosen from the group consisting of restenosis, coronary artery disease, atherosclerosis, atherogenesis, cerebrovascular disease, angina, ischemic disease, congestive heart failure, pulmonary edema associated with acute myocardial infarction, thrombosis, high or elevated blood pressure in hypertension, platelet aggregation, platelet adhesion, smooth muscle cell proliferation, a vascular or nonvascular complication associated with the use of a medical device, a wound associated with the use of a medical device, vascular or nonvascular wall damage, peripheral vascular disease, neointimal hyperplasia following percutaneous transluminal coronary angiograph, and a combination thereof.

51. The method of claim 47 wherein the recipient subject has a pathological condition resulting from abnormal cell proliferation.

52. The method of claim 47 wherein the recipient subject has a disease selected from the group consisting of a cancer, a transplant rejection, an autoimmune disease, an inflammatory disease, a proliferative disease, a hyperproliferative disease, a vascular disease, a scar tissue, a wound contraction, and a combination thereof.

53. The method of claim 47 wherein the recipient subject has a pathological condition resulting from abnormal cell adherence.

54. The method of claim 47 wherein the dendritic nitric oxide donor further comprises a targeting agent.

55. The method of claim 47 wherein the dendritic nitric oxide donor further comprises a guest molecule.

56. The method of claim 47 wherein the dendritic nitric oxide donor further comprises a guest molecule chosen from the group consisting of a drug, a therapeutic agent, a diagnostic agent, and a combination thereof.

57. The method of claim 47 wherein the amount of dendritic nitric oxide donor is sufficient to provide a therapeutic amount of NO to the recipient subject.

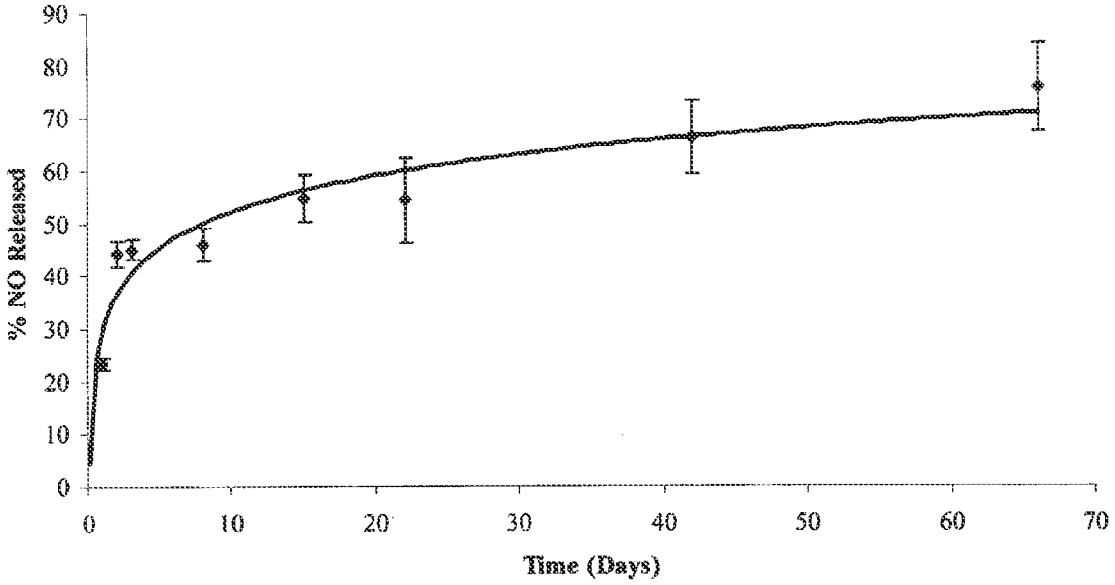


FIGURE 1.

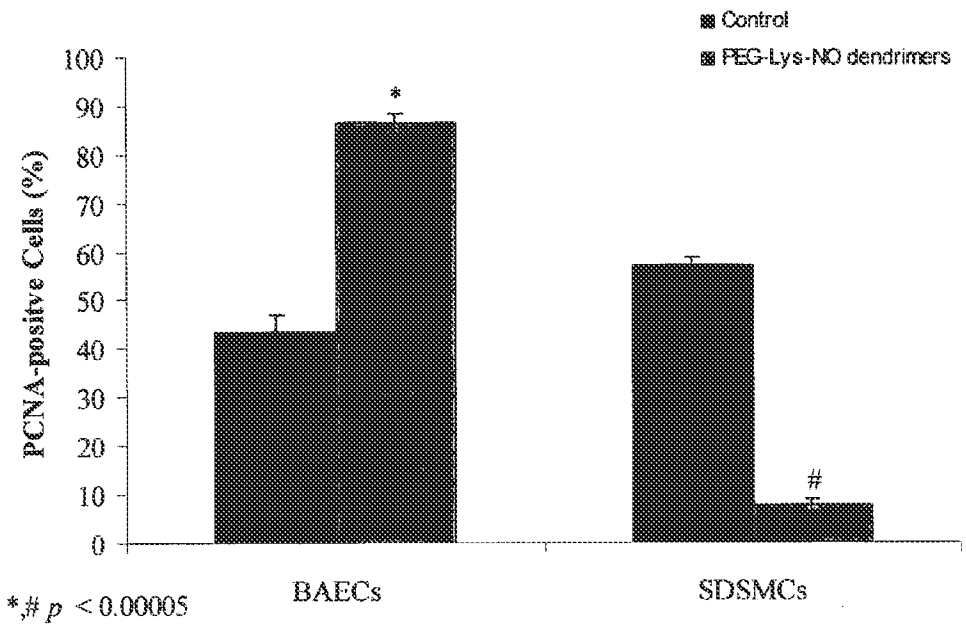

Control
PEG-Lys-NO dendrimers
PCNA-positive Cells (%)
100
90
80
70
60
50
40
30
20
10
0
*
#
BAECs
SDSMCs
*,# p < 0.00005

FIGURE 2.

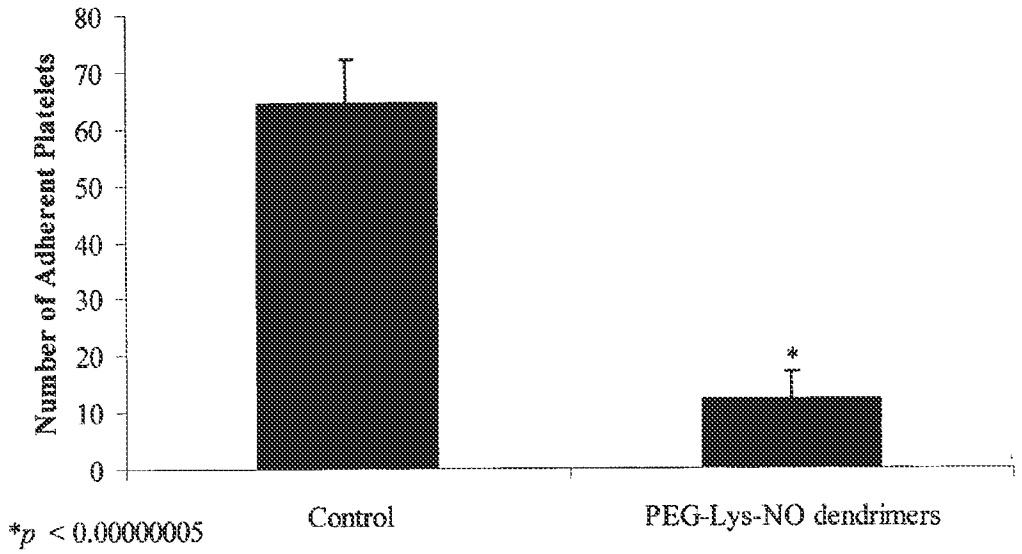

Number of Adherent Platelets
80
70
60
50
40
30
20
10
0
*
Control
PEG-Lys-NO dendrimers
*p < 0.00000005

FIGURE 3.

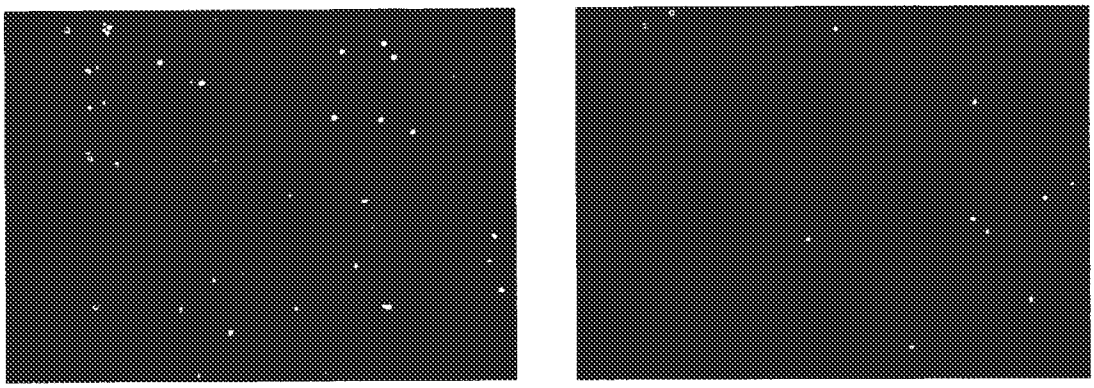


FIGURE 4.

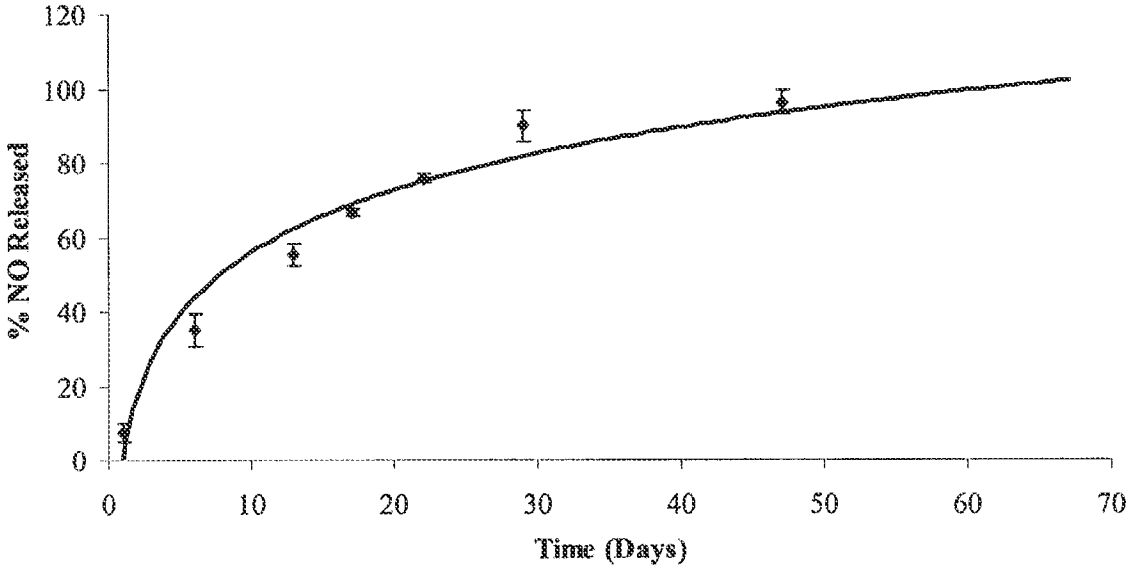


FIGURE 5.

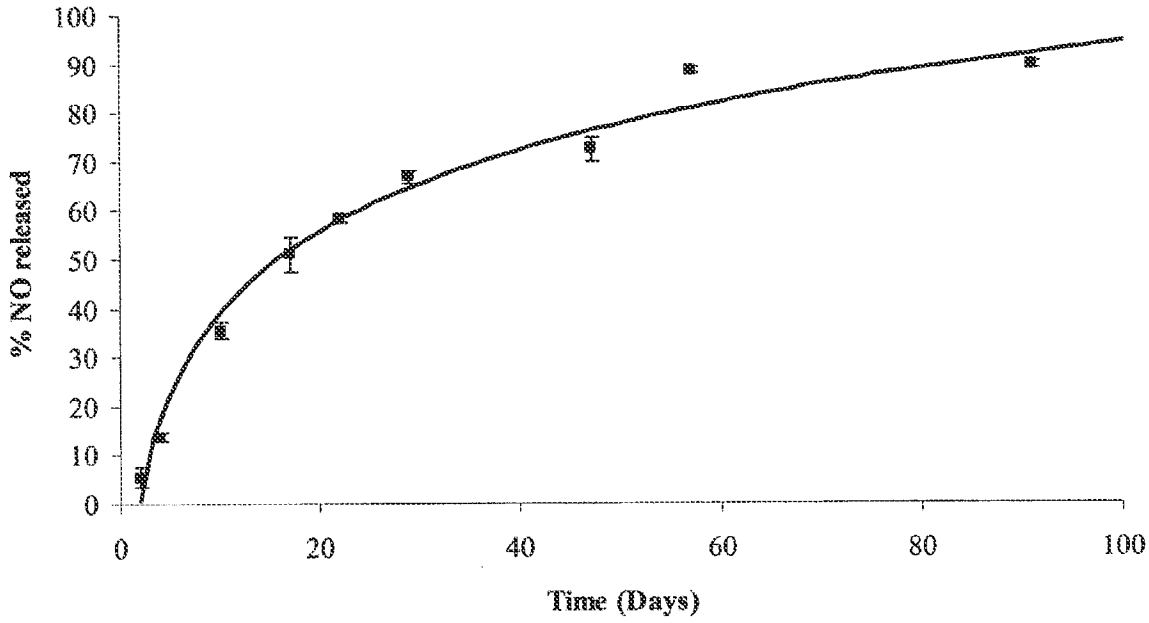

100
90
80
70
60
50
40
30
20
10
0
% NO released
0
20
40
60
80
100
Time (Days)

FIGURE 6.

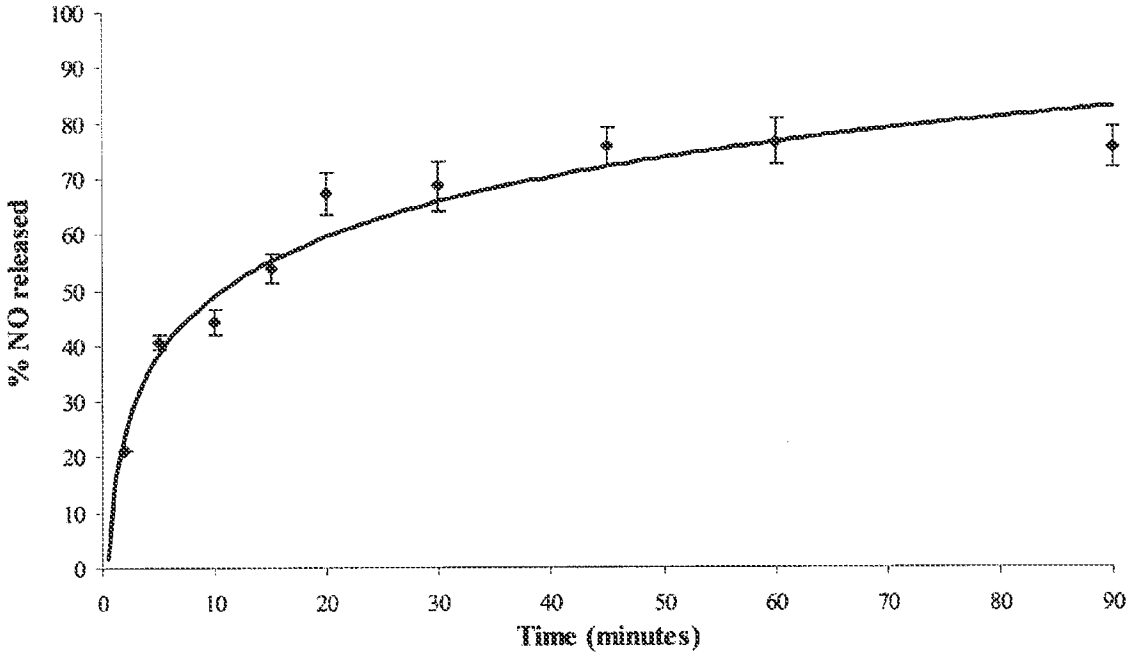


FIGURE 7.

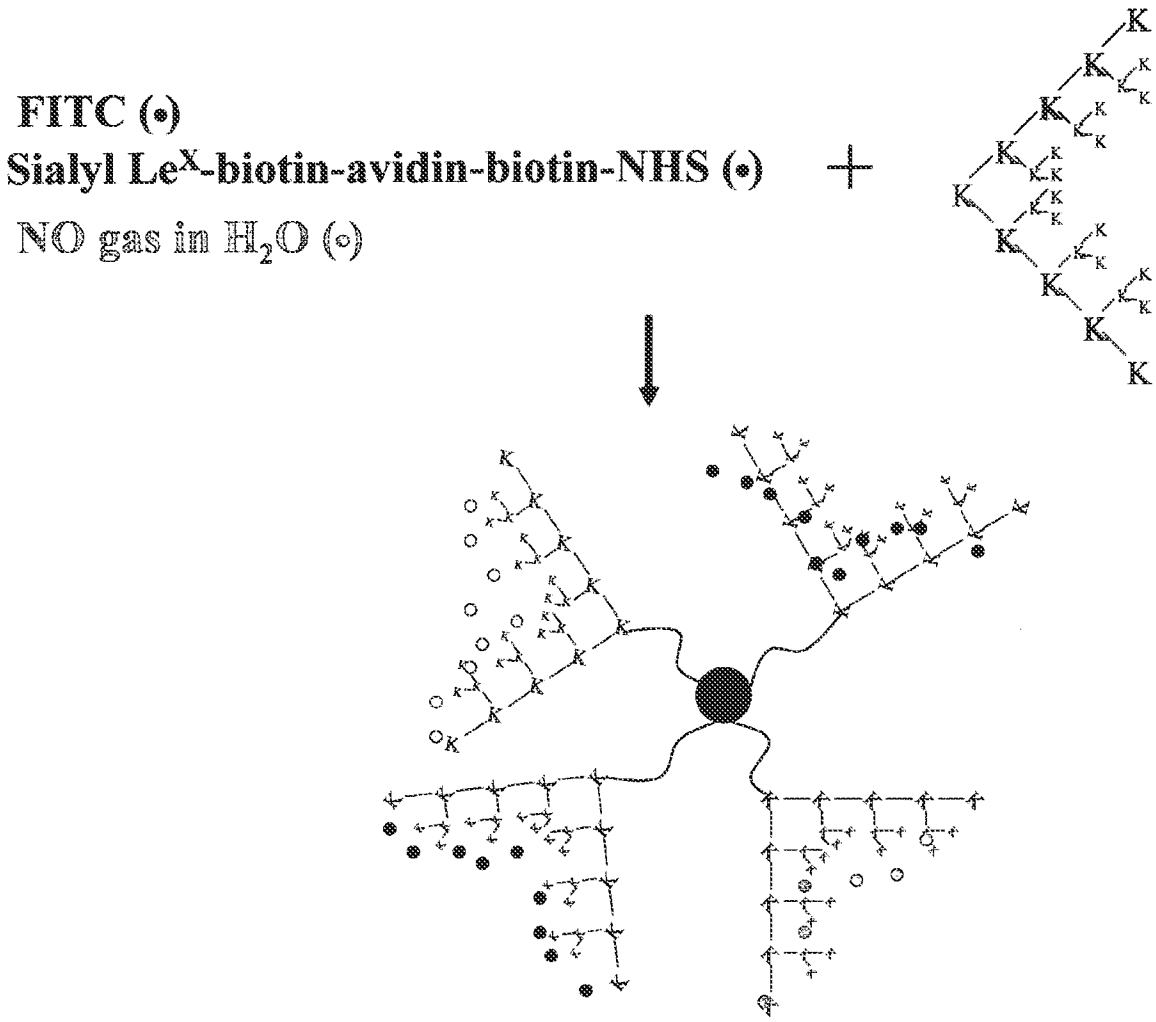
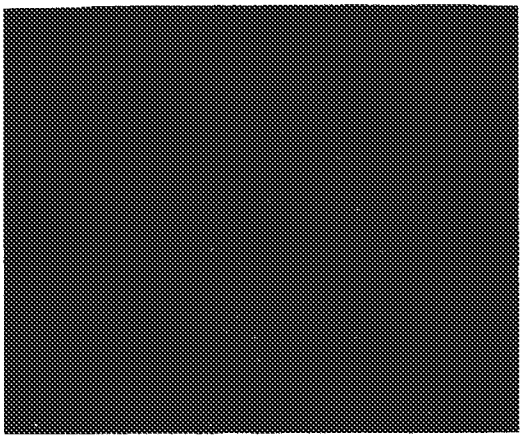
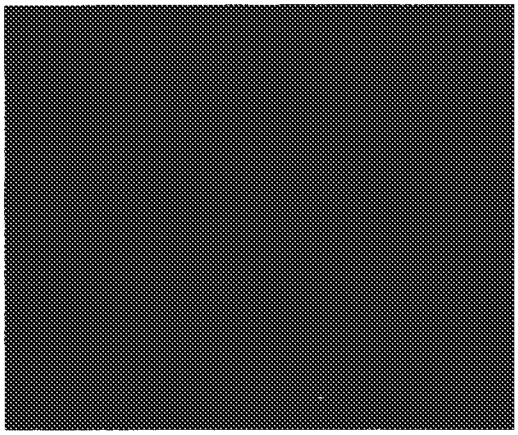


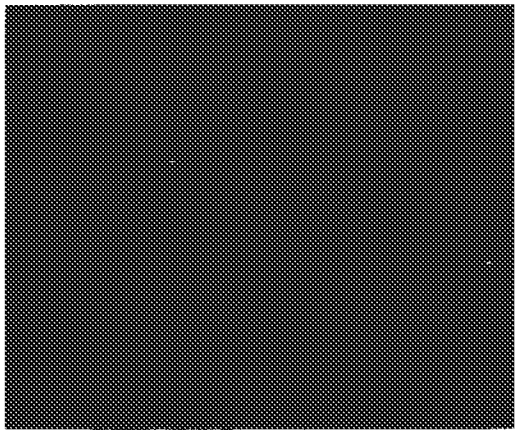
FIGURE 8.



A



B



C

FIGURE 9.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/17056

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/74
US CL : 424/78.27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/78.27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,379,691 B1 (TADESCHI et al) 30 April 2002 (30.04.2002), see the whole document.	1-57
A	US 6,660,034 B1 (MANDRUSOV et al) 09 December 2003 (09.12.2003), see the whole document.	1-57

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 September 2005 (06.09.2005)	27 SEP 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer BLESSING FUBARA Telephone No. 571-272-1600

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